Kidney International, Vol. 42 (1992), pp. 25–32

Immunocytochemical study of glomerular permeability to anionic, neutral and cationic albumins

LUCIAN GHITESCU, MICHEL DESJARDINS, and MOÏSE BENDAYAN

Département d'anatomie, Faculté de médecine, Université de Montréal, C.P. 6128, succursale A, Montréal, Québec, Canada

Immunocytochemical study of glomerular permeability to anionic, neutral and cationic albumins. The renal handling of albumin of various isoelectric points (pI) was studied in mice by high resolution quantitative immunocytochemistry. Bovine serum albumin (BSA) was tagged with dinitrophenol (DNP) and cationized to pI 6.5 to 7.5 and to pI over 8.5. These tracers, including the anionic BSA-DNP (pI 4.8) were injected in the iliac vein of mice and the post-embedding protein A-gold technique was used with antibodies against DNP to localize the different tracers in renal tissue. Morphometric analysis of the labeling over the glomerular wall has demonstrated variations in its distribution according to the pI of the markers. Anionic and neutral BSA molecules were detected on the endothelial side of the basement membrane, while labeling for cationic BSA was present mainly in the laminae rarae interna and externa known to display fixed polyanionic sites. Neutral and cationic BSA-DNP reached the urinary space and were detected within the endocytotic apparatus of the proximal tubule epithelium. Neutralization of the anionic sites of the basement membrane with cationic BSA resulted in an increase in the permeability towards anionic BSA-DNP. In addition to the demonstration of glomerular permeability properties towards various probes, the present study has demonstrated that dinitrophenylated albumin represents an excellent versatile tool for the quantitative morphological investigation of glomerular permeability.

The glomerular basement membrane acts as a filtration barrier restricting the passage of serum proteins according to their physico-chemical properties [1, 2]. Tracers such as dextrans, ferritin and their derivatives of various charges have been used in previous studies to demonstrate that size as well as net electrical charges of circulating molecules play crucial roles in renal filtration [3–10]. It has been demonstrated that the organization of the collagenous lattice of the glomerular basement membrane is an important determinant regulating the permselective properties [11]. On the other hand, polyanionic constituents of the basement membrane have been shown to be directly involved in restricting the passage of anionic circulating proteins by creating a charge selective barrier [12]. The nature of this electrostatic screen is at present a matter of controversy. While some data suggest that the main role is played by the heparan sulfate proteoglycan [13], challenging evidences indicate that ionized carboxyl groups other than those belonging to hyaluronic or neuraminic acids might be the key components [14, 15]. Whatever the biochemical substrate of this electrostatic barrier might be, it favors the passage of cationic proteins, while preventing the egress of the anionic ones.

It has also been suggested that the mentioned fixed polyanions detain important roles in the preservation of the porosity of the glomerular basement membrane, since neutralization of these charges leads to the passage of large anionic as well as neutral macromolecules which normally do not reach the urinary space [14, 16].

This current view on glomerular permeability emerged from two types of investigation, complementary to each other: morphologic ultrastructural observations using, however, non-physiological electron opaque tracers of different sizes and charges, and experiments of physiology, usually employing radioactively-tagged plasma proteins, but lacking a direct ultrastructural support. In the present investigation we have assessed the usefulness of quantitative immunocytochemistry in the study of the renal glomerular permeability to plasma macromolecules. As a model we have chosen serum albumin of different isoelectric points (pI) which has been covalently tagged with dinitrophenol (DNP). This procedure facilitates the immunodetection of the dinitrophenylated albumin molecules (BSA-DNP) in tissues fixed with aldehydes and OsO_4, by using specific antibodies to DNP and protein A-gold [17]. The approach allows for a direct visualization, at the ultrastructural level, of the charge selectivity of glomerular basement membrane towards albumin molecules of various pI, which has only been previously documented by physiological measurements [18, 19].

Methods

Bovine serum albumin (BSA) crystallized and lyophilized, ethylenediamine and 1-ethyl-3-(3 dimethylaminopropyl) carbodiimide (EDC) were purchased from Sigma Chemical Co. (St. Louis, Missouri, USA). 2,4-Dinitrobenzene sulfonic acid was from Aldrich Chemical Co. (Milwaukee, Wisconsin, USA), and anti-dinitrophenol antibodies (rabbit IgG purified fraction) were from Dako Corporation (Carpinteria, California, USA).

For in situ immunodetection, BSA was tagged with dinitrophenol according to the method of Little and Eisen [20] as described in detail previously [17]. Spectrophotometrically, an average of 7.5 DNP residues were found to be coupled to each BSA molecule.

Dinitrophenylated BSA was used as such or was modified by substituting some of its carboxyl groups with amino residues,

Received for publication August 1, 1991
and in revised form February 20, 1992
Accepted for publication February 22, 1992

reacting them with carbodiimide in the presence of a diamine as a nucleophile [21]. The degree of substitution was controlled by performing the reaction at fixed BSA-DNP/EDC/ethylenediamine ratios and at different pH values ranging between 4.7 to 7.0. Cationic BSA-DNP derivatives of various isoelectric points (pI) were thus obtained, extensively dialyzed against 0.01 M phosphate buffered saline (PBS) pH 7.2 and brought to a final concentration of 20 mg/ml by ultrafiltration through a PM-30 membrane (Amicon, Danvers, Massachusetts, USA). The isoelectric point of the modified albumins was determined by flat bed isoelectric focusing against various pI markers (Pharmacia Fine Chemical Co., Uppsala, Sweden) in 1% agarose IEF and pH 3.5 to 10.0 Pharmalytes.

CD-1 male mice, weighing 35 to 40 g, fed ad libitum, were used for the experiments. From the common iliac vein of ether anaesthetized animals, 0.3 ml of blood was withdrawn and 0.4 ml of one of the tracers (prewarmed at 37°C) was injected. The whole procedure lasted two to three minutes and the animals recovered immediately. Thirty minutes after injection, the mice were sacrificed by cervical dislocation, the abdominal cavity was opened and flooded with 1% glutaraldehyde in 0.1 M phosphate buffer. The kidneys were then cut into small blocks, further fixed in the glutaraldehyde solution for one hour, post-fixed with 1% OsO_4 and embedded in Epon 812 according to the standard procedures. Thin sections were cut and mounted on nickel grids coated with a film of Parlodion and carbon. The protein A-gold post-embedding technique [22] was used together with polyclonal antibodies (IgG fraction) against DNP to reveal the various types of BSA-DNP within the renal tissues. Briefly, the tissue sections were incubated for 15 minutes in a 1% solution of ovalbumin in PBS, and transferred to a drop of the antibody solution for 60 minutes at room temperature. The antibody was diluted 1/1000 with PBS containing 1% ovalbumin. The grids were then washed in PBS and transferred to a drop of protein A-gold (15 nm particle diameter) for 30 minutes at room temperature. The grids were then washed with distilled water, dried and stained with uranyl acetate and lead citrate, prior to the observation with a Philips 410 electron microscope.

For the evaluation of the dynamics of the glomerular filtration, in some experiments, the animals were sacrificed at 10 minutes, two hours or five hours after the injection of the tracers. To test the influence of a circulating cationic molecule on the glomerular filtration of an anionic tracer, anionic BSA-DNP (pI 4.8) was injected together with a non-dinitrophenylated cationized BSA (pI 8.5). Thirty minutes after the injection, the kidneys were fixed and processed as described.

The distribution of the labeling over the thickness of the glomerular basement membrane was evaluated for each kind of tracer as previously described [23–25], on images recorded at 16900 magnification and analyzed using a Videoplan 2 system (Carl Zeiss, Toronto, Ontario, Canada). The distance d from the abluminal endothelial plasma membrane to the gold particles and the thickness T of the glomerular basement membrane at the same site were measured. Their ratio R = d/T was used to express the relative position of each detected BSA-DNP molecule across the basement membrane. Three animals were used for each of the experimental condition. For each animal, three sections were immunocytochemically processed, an average of six glomeruli were morphometrically examined and an aggregated length of 120 μm glomerular basement membrane was screened for establishing the gold particle distribution. Data corresponding to each type of tracer were pooled and expressed in a single histogram depicting the percentage of gold particles having a certain localization across the glomerular basement membrane.

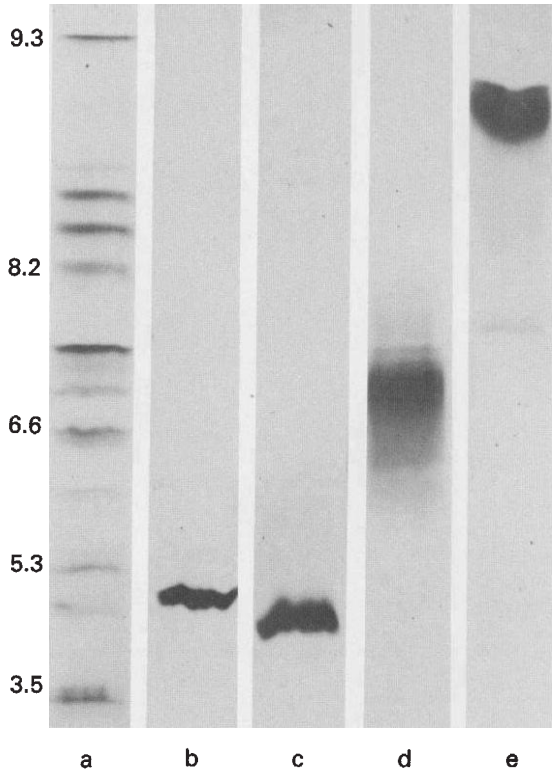

Fig. 1. *Isoelectric focusing of BSA-DNP derivatives.* pI markers (lane a); native BSA (lane b); anionic BSA-DNP, pI 4.8 (lane c); neutral BSA, pI 6.5 to 7.5 (lane d); cationic BSA-DNP, pI over 8.5 (lane e). Twenty μg of albumin loaded on each lane.

The specificity of the immunodetection for the DNP residues was assessed by performing the post-embedding immunolabeling procedure on specimens derived from animals which did not receive any tracer or which were injected with non-dinitrophenylated, native albumin. Additionally, 5 μg of native BSA, BSA-DNP, non-dinitrophenylated cationic BSA and cationized BSA-DNP were adsorbed in spots on nitrocellulose membrane, air dried and sequentially incubated with anti-DNP (1/1000), biotinylated goat anti-rabbit IgG and avidin-HRP (Vectastain ABC kit, Vector Lab., Burlingame, California, USA) according to manufacturer's instructions for nitrocellulose-bound antigen detection. As a quencher for nonspecific binding, all solutions contained 0.1% Tween 20. The substrate used for the peroxidatic reaction was 4-chloro-1-naphtol.

Results

The measurement of the pI of the various markers by isoelectric focusing on agarose gel (Fig. 1) shows the presence of BSA-DNP bands at pI around 4.8 (designated as anionic BSA-DNP) close to that of native BSA, at pI between 6.5 and

7.5 (designated as neutral BSA-DNP), and at pI over 8.5 (designated as cationic BSA-DNP).

Thirty minutes after intravenous injection of the tracers, the labeling for anionic BSA-DNP (Fig. 2a) was present within the capillary lumen and in the glomerular basement membrane, mainly on its endothelial aspect, few gold particles being only observed on the epithelial side. This was confirmed by the morphometrical analysis of the distribution of this tracer over the thickness of the basement membrane. Under the same experimental conditions, neutral BSA-DNP (Fig. 2b) was found to penetrate deeper in the thickness of the glomerular basement membrane and to reach the epithelial side. Indeed, the analysis of its ultrastructural distribution shows a high percentage of labeling in the endothelial side of the basement membrane and an increase of the neutral tracer in the region close to the epithelial cell. In contrast, cationic BSA-DNP (Fig. 2c) was found over the entire thickness of the glomerular basement membrane, being particularly highly concentrated in the laminae rare interna and externa. This is again confirmed by the morphometrical analysis showing peaks of labeling in the regions close to the endothelial and epithelial cells. Labelings for each of the tracers, including the anionic (Fig. 3a) and cationic (Fig. 3b) BSA-DNP, were also observed in the extracellular matrix of the mesangial area. The labelings did not seem to display particular or different distributions according to the various tracers. However, the cationic BSA-DNP yielded a higher labeling when compared to the anionic or neutral ones. Only few gold particles have been detected over the endothelial, epithelial and mesangial cell profiles, indicating a low endocytic activity of these cells for the tracers, irrespective of their charge. In contrast, the neutral and particularly the cationic BSA-DNP (Fig. 4), upon crossing the glomerular basement membrane, yielded high intensities of labeling in the epithelial cells of nearly all proximal tubules examined. The tracers were present in tubulo-vesicular structures in the apical region of the cells as well as in large endocytic vesicles and lysosomes (Fig. 4).

To assess the dynamics of the passage of various albumin tracers through the glomerular basement membrane, the anionic and cationic BSA-DNP were injected and traced in the glomerular wall at time intervals ranging from 10 minutes to five hours after injection. Basically, no variations in the pattern of labeling were observed for each of the tracers between 10 and 30 minutes of circulation time, demonstrating that the equilibration between the circulating proteins and the glomerular basement membrane is a rapid process. However, at longer time points the presence of the tracers in the kidney varied significantly according to their charge. The anionic BSA-DNP was still recorded in high concentrations within the capillary lumen and maintained the initial profile in the glomerular basement membrane for up to five hours after the injection (Fig. 5a). Instead, the labeling for cationic BSA-DNP disappeared gradually from the blood and from the glomerular basement membrane within the first two hours (Fig. 5b).

To investigate the role of anionic sites of the glomerular basement membrane in restricting the passage of anionic albumin, cationic non-dinitrophenylated BSA and anionic BSA-DNP were injected simultaneously. Consequently, anionic BSA-DNP was detected across the entire thickness of the glomerular basement membrane (Fig. 6a), at variance with the pattern obtained when the tracer was injected alone (Fig. 2a). The morphometric analysis further confirmed the ability of cationized albumin to increase the glomerular permeability to its anionic variant; a homogenous distribution of anionic BSA-DNP labeling was recorded throughout the glomerular basement membrane.

The reaction of anti-DNP antibodies with nitrocellulose-bound BSA, cationized BSA and their dinitrophenylated derivatives has clearly demonstrated that only DNP residues are recognized by the immunoreagents (Fig. 7). Moreover, no post-embedding immunocytochemical signal was detected in specimens harvested from animals which did not receive any tracer or which were injected with BSA only (figures not shown). This indicates that the anti-DNP immunoglobulin used does not recognize either the endogenous or the exogenous non-dinitrophenylated albumins, being highly specific only for DNP-tagged molecules.

Discussion

Native anionic BSA and its neutral and cationic derivatives were tagged to DNP molecules, injected intravenously to mice, and localized in the renal cortex by immunocytochemistry. This has enabled direct observations on how the glomerular wall handles plasma proteins such as albumin carrying different electric charges. The use of DNP as a tag for serum proteins allows for their detection against the endogenous species, in tissues displaying optimal ultrastructural preservation. Renal permeability can therefore be studied at the electron microscopical level of resolution, without using non-physiological or particulate probes, but rather tracing macromolecules of physiological significance. Moreover, the use of the post-embedding protein A-gold technique, in addition to the high level of resolution, provides the opportunity for a quantitative morphometrical analysis of the tracer distribution in various compartments of the glomerular wall. This might be of particular importance in the fine study of morpho-functional alterations in various renal pathological circumstances [23], allowing for a separate examination of the filtration and reabsorption processes.

The validity of this approach is strengthened by the fact that our results go along with the physiological studies of renal permeability to albumins of different electric charges [18, 19]. The handling of the different probes by the glomerular basement membrane did vary according to their charges. The passage of the anionic marker was by far more efficiently restricted than that of its derivative cationized counterparts, as suggested by three lines of evidence: anionic BSA-DNP remained in circulation for longer periods of time, its labeling was confined to the endothelial side of the glomerular basement membrane, and very few molecules were detected in the proximal tubules. This pattern of distribution is identical with that obtained for endogenous albumin [11, 23] and agrees with the current models explaining the mechanism of renal permeability. According to these, electrostatic barriers made of intrinsic anionic sites localized in the lamina rara interna and externa [13, 24] repels most of the anionic plasma molecules such as albumin. In between, the meshwork of lamina densa might act as a size-exclusion filter, which would explain why the endogenous anionic (albumin) or neutral (immunoglobulins) molecules are almost completely arrested at this level [11, 23, 25]. In contrast,

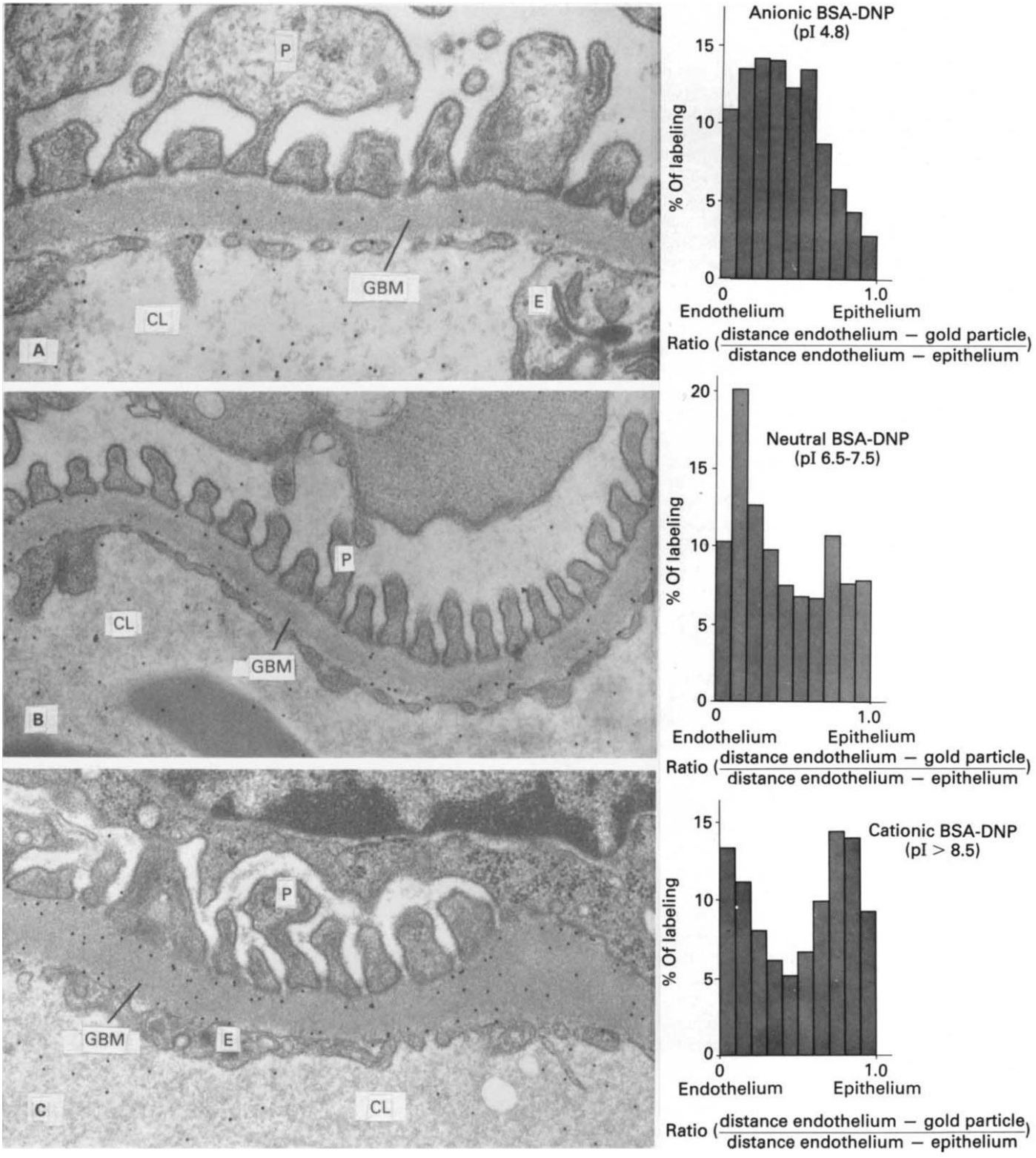

Fig. 2. *Localization of anionic (a), neutral (b), and cationic (c) BSA-DNP in the glomerular wall of mice, 30 minutes after intravenous injection.* Anionic BSA-DNP is present on the endothelial side of the glomerular basement membrane (GBM), whereas few gold particles reach the epithelial side (a). This is further demonstrated by the accompanying histogram (a). Neutral BSA-DNP is present mainly on the lamina rara interna. The lamina densa is also labeled (b). Cationic BSA-DNP labeling is present preferentially in both lamina rarae (c). This is further demonstrated by the histogram (c). CL, capillary lumen; E, endothelial cell; P, podocyte. a = 38,800×; b, c = 33,950×.

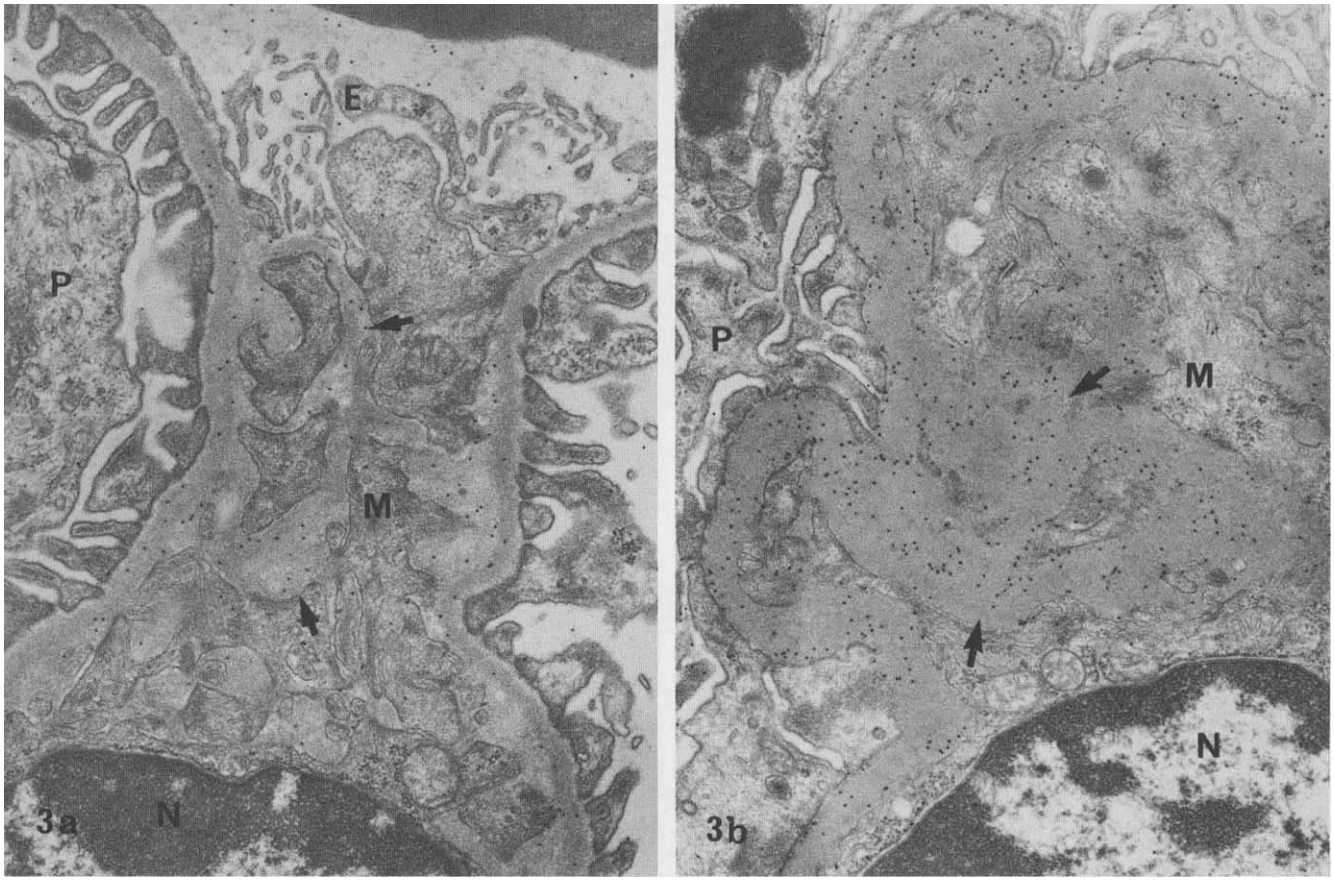

Fig. 3. *Localization of anionic (a) and cationic (b) BSA-DNP in the mesangial region, 30 minutes after the injection.* The labeling is present over the extracellular matrix (arrows). Few gold particles over the mesangial cell (M). E, endothelial cell; N, nucleus of the mesangial cell; P, podocyte. a, b = 19,000×.

cationized BSA-DNP crossed the basement membrane and labeled, during its transit, both laminae rarae, most probably due to the presence of intrinsic anionic sites. The labeling distribution obtained for cationic BSA-DNP corresponds to that reported for heparan sulfate proteoglycans [24, 26]. For neutral albumin, in spite of the fact that a peak of labeling was still found on the endothelial side of the glomerular basement membrane, a significantly larger proportion of the molecules reached the epithelial side. The basement membrane appears to be quite permeable to neutral BSA-DNP since neutral BSA-DNP as well as the cationic derivative were found in many epithelial cells of the proximal tubules. The fact that the glomerular basement membrane allows the passage of cationized, and to a certain extent of neutral albumin but not of the identically-sized anionic molecule suggests that the charge-selective barrier is the dominant element in preventing the escape of native albumin. An alternative, which still remains to be demonstrated, implies that the tracer itself could alter the porosity of the filter believed to be represented by the lamina densa [14].

The experimental condition in which cationic BSA and anionic BSA-DNP were injected simultaneously resulted in the passage of anionic BSA-DNP through the entire glomerular basement membrane. This is in sharp contrast with the lack of glomerular permeability for anionic BSA-DNP when injected alone. These results together with those published previously [27–29] demonstrate that neutralization of the anionic sites of the glomerular basement membrane by cationic molecules results in an increase of permeability. Interestingly, in pathological conditions such as diabetes, a "cationization" of albumin has been described [30]. It is possible that such a modified albumin binds to basement membrane polyanions, neutralizes them and induces the loss of the charge selectivity of the glomerular wall.

Previous studies using cationized ferritins have demonstrated an increased penetration of the glomerular basement membrane by these probes, which appears to be related to the degree of cationization [9, 13, 31, 32]. Our results with albumin and its derivatives generally agree with these data, but they also display some differences. Whereas cationic ferritin accumulates and labels in a rather repetitive pattern distinct sites in the laminae rarae interna and externa [13, 31, 32], cationic BSA-DNP appeared to be distributed more homogeneously in these regions. Also at variance with the positively charged ferritins, cationic BSA-DNP was detected within the lamina densa. Previous experiments have shown that cationic ferritin is retained in the glomerular basement membrane for as long as 72 hours [31] after injection. In contrast, cationic BSA-DNP was

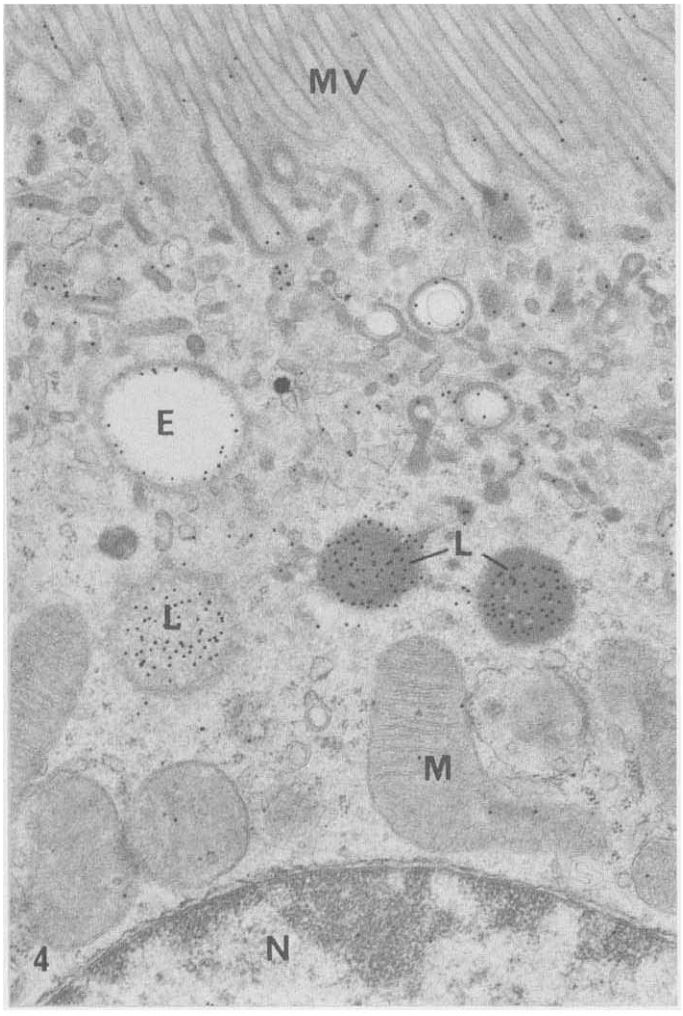

Fig. 4. *Localization of cationic BSA-DNP in an epithelial cell of the proximal tubule, 30 minutes after the injection.* The labeling is present on the endocytic structures on the apical side of the cell, including endosome-like (E) and lysosome-like (L) structures. M, mitochondria; MV, microvilli; N, nucleus of the epithelial cell. 21,000×.

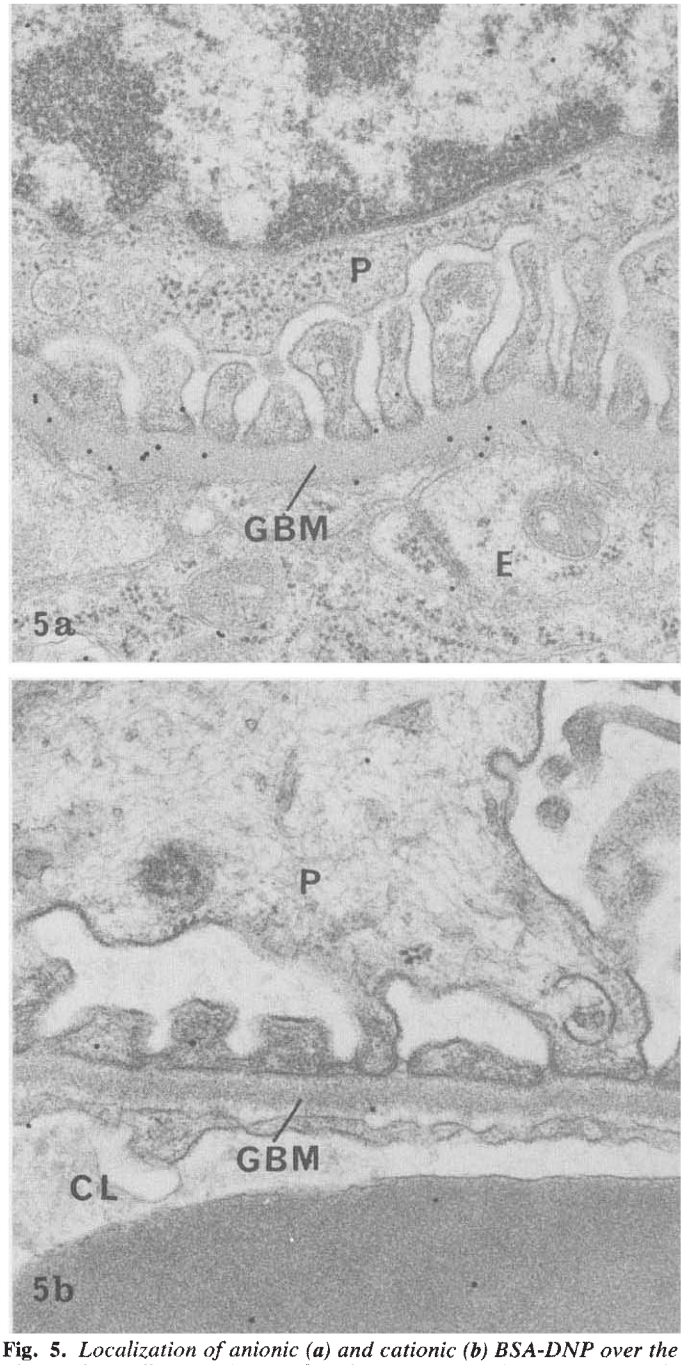

Fig. 5. *Localization of anionic (a) and cationic (b) BSA-DNP over the glomerular wall at two hours after the injection.* Anionic BSA-DNP is still present on the endothelial side of the glomerular basement membrane (GBM) as demonstrated by the labeling (a). Cationic BSA-DNP is cleared from the capillary lumen (CL) and the basement membrane as demonstrated by the low labeling obtained (b). E, endothelial cell; P, podocyte. a, b = 37,000×.

cleared from the circulation and disappeared from the glomerular basement membrane within two hours. Much larger in size, ferritin could be trapped and retained in the basement membrane for longer periods of time. Indeed, previous studies have demonstrated clogging of glomerular basement membrane by native ferritin after neutralization of the anionic sites [12]. In contrast, none of our experimental conditions using the various BSA markers resulted in the clogging of the basement membrane. It thus appears that albumin tagged to dinitrophenol is an interesting and more physiological marker for the study of glomerular permeability. Dinitrophenylation as a tagging procedure can be extended to other plasma macromolecules in order to investigate their handling by the kidney in normal as well as in pathological conditions.

Acknowledgments

This study was supported by grants from the Medical Research Council of Canada, from which L. Ghitescu and M. Desjardins are recipients of Studentship awards. The authors thank Diane Gingras, Jean Godbout, Emilienne Lambert and Jean Léveillé for their technical assistance.

Reprint requests to Dr. Moïse Bendayan, Département d'anatomie, Université de Montréal, C.P. 6128, Succ. A, Montréal, Québec, Canada, H3C 3J7.

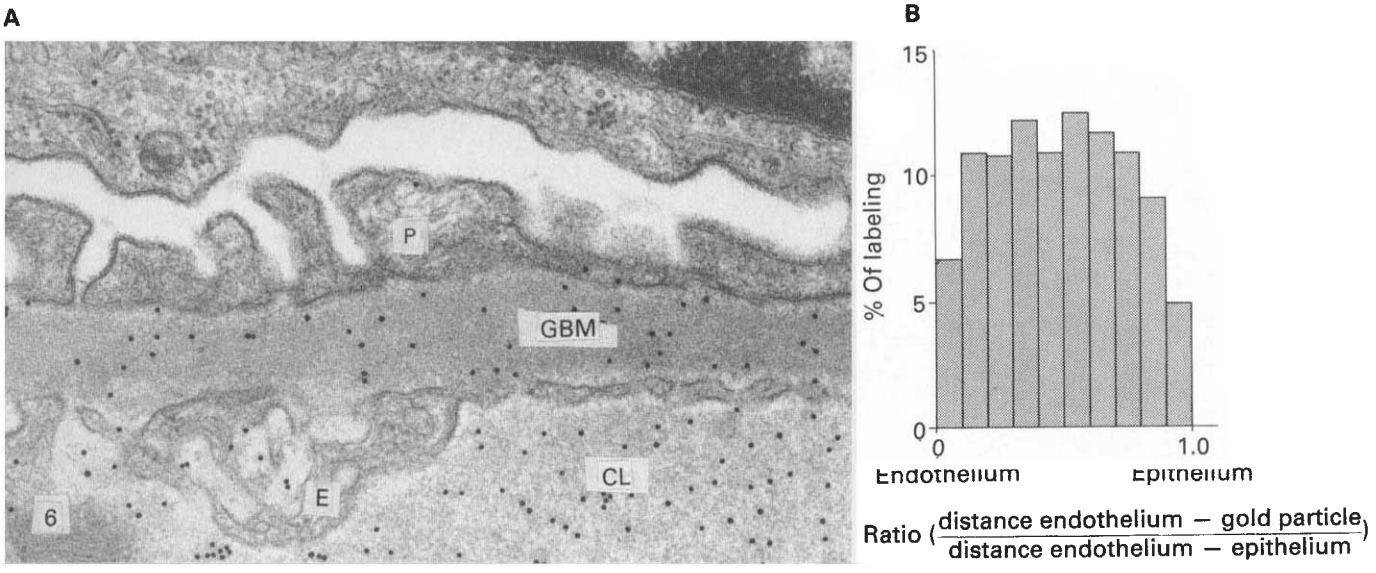

Fig. 6. *Localization of anionic BSA-DNP over the glomerular wall when co-injected with cationic BSA, 30 minutes after the injection.* The labeling is present on the entire thickness of the glomerular basement membrane (GBM). This is further demonstrated by the accompanying histogram. CL, capillary lumen; E, endothelial cell; P, podocyte. 42,000×.

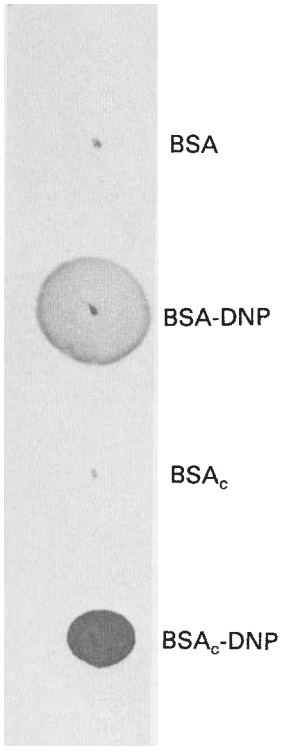

Fig. 7. *Dot blot immunodetection of native BSA, BSA-DNP, cationized, non-dinitrophenylated BSA (BSA$_c$) and its dinitrophenylated derivative (BSA$_c$-DNP) using anti-DNP antibodies.* Each spot contains 5 μg of each antigen. Positive signal is obtained for BSA-DNP and cationized BSA-DNP only.

References

1. Brenner BM, Hostetter TH, Hulmes HD: Glomerular permselectivity: barrier function based on discrimination of molecular size and charge. *Am J Physiol* 234:F455–F460, 1978
2. Farquhar MG: The glomerular basement membrane. A selective macromolecular filter, in *Cell Biology of Extracellular Matrix*, edited by Hay ED, New York, Plenum Press, 1981, pp. 335–378
3. Pappenheimer JR: Passage of molecules through capillary walls. *Physiol Rev* 33:387–423, 1953
4. Wallenius G: Renal clearance of dextran as a measure of glomerular permeability. *Acta Soc Med Uppsal* 59:1–91, 1954
5. Farquhar MG, Wissig SL, Palade GE: Glomerular permeability I. Ferritin transfer across the normal glomerular capillary wall. *J Exp Med* 113:47–66, 1961
6. Caulfield JP, Farquhar MG: The permeability of glomerular capillaries to graded dextrans. Identification of the basement membrane of the capillaries as the primary filtration barrier. *J Cell Biol* 63:883–903, 1974
7. Chang RLS, Deen WM, Robertson CR, Brenner B: Permselectivity of the glomerular capillary wall. III. Restricted transport of polyanions. *Kidney Int* 8:212–218, 1975
8. Rennke HG, Cotran RS, Venkatachalam MA: Role of molecular change in glomerular permeability: Tracer studies with cationized ferritins. *J Cell Biol* 67:638–646, 1975
9. Rennke HG, Venkatachalam MA: Glomerular permeability: *In vivo* tracer studies with polyanionic and polycationic ferritins. *Kidney Int* 11:44–53, 1977
10. Rennke HG, Patel Y, Venkatachalam MA: Effect of molecular charge on glomerular permeability to proteins in the rat: Clearance studies using neutral, anionic and cationic horseradish peroxidase. *Kidney Int* 13:278–288, 1978
11. Desjardins M, Bendayan M: Ontogenesis of glomerular basement membrane: structural and functional properties. *J Cell Biol* 113:689–700, 1991
12. Kanwar YS, Rosenzweig LJ: Clogging of the glomerular basement membrane. *J Cell Biol* 93:489–494, 1982
13. Kanwar YS, Farquhar MG: Anionic sites in the glomerular basement membrane. *In vivo* and *in vitro* localization to the laminae rarae by cationic probes. *J Cell Biol* 81:137–153, 1979
14. Hunsicker LG, Bertolatus JA: Charge compounds of the glomerular filter and their role in normal and disordered permselectivity. *Artif Organs* 11:468–477, 1987
15. Bray J, Robinson GB: Influence of charge on filtration across renal basement membrane films *in vitro*. *Kidney Int* 25:527–533, 1984
16. Barnes JL, Radnik RA, Gilchrist EP, Venkatachalam MA:

Size and charge selective permeability defects induced in glomerular basement membrane by a polycation. *Kidney Int* 25:11–19, 1984
17. GHITESCU L, BENDAYAN M: Transendothelial transport of serum albumin. A quantitative immunocytochemical study. *J Cell Biol* (in press)
18. PURTELL JN, PESCE AJ, CLYNE DH, MILLER WC, POLLAK VE: Isoelectric point of albumin: Effect on renal handling of albumin. *Kidney Int* 16:366–376, 1979
19. GINEVRI F, GHIGGERI GM, CANDIANO G, OLEGGINI R, BERTELLI R, PERFUMO F, QUEIROLO C, GUSMANO R: Endogenous albumin as a marker of renal selectivity in steroid-unresponsive nephrotic syndrome. *Nephron* 52:133–138, 1989
20. LITTLE JR, EISEN HN: Preparation of immunogenic 2,4 dinitrophenyl and 2,4,6 trinitrophenyl proteins. *Meth Immunol Immunochem* 1:128–133, 1967
21. HOARE DG, KOSHLAND DE: A method for the quantitative modification and estimation of carboxylic acid groups in proteins. *J Biol Chem* 242:2447–2453, 1967
22. BENDAYAN M: Protein A-gold electron microscopic immunocytochemistry: Methods, applications and limitations. *J Elec Micr Tech* 1:243–270, 1984
23. BENDAYAN M, GINGRAS D, CHAREST P: Distribution of endogenous albumin in the glomerular wall of streptozotocin-induced diabetic rats as revealed as high-resolution immunocytochemistry. *Diabetologia* 29:868–875, 1986
24. STOW JL, SAWADA H, FARQUHAR MG: Basement membrane heparan sulfate proteoglycans are concentrated in the laminae rarae and in podocytes of the rat renal glomerulus. *Proc Natl Acad Sci USA* 82:3296–3300, 1985
25. DESJARDINS M, BENDAYAN M: Ultrastructural distribution of endogenous IgGs in the glomerular wall of control and diabetic rats. *Histochem J* 21:731–742, 1989
26. KERJASCHKI D, SAWADA H, FARQUHAR MG: Immunoelectron microscopy in kidney research: Some contributions and limitations. *Kidney Int* 30:229–245, 1986
27. FIRTH JD: Effect of polycations on the function of the isolated perfused rat kidney. *Clin Sci* 79:591–598, 1990
28. HUNSICKER LG, SHEARER TP, SHAFFER SJ: Acute reversible proteinuria induced by infusion of the polycation hexadimethrine. *Kidney Int* 20:7–17, 1981
29. VEHASKARI VM, ROOT ER, GERMUTH FG JR, ROBSON AM: Glomerular charge and urinary protein excretion: Effects of systemic and intrarenal polycation infusion in the rat. *Kidney Int* 22:127–135, 1982
30. GHIGGERI GM, CANDIANO G, DELFINO G, QUEIROLO C: Electrical charge of serum and urinary albumin in normal and diabetic humans. *Kidney Int* 28:168–177, 1985
31. COHEN S, VERNIER RL, MICHAEL AF: The effect of charge on the renal distribution of ferritin. *Am J Pathol* 110:170–181, 1983
32. BLISS DJ, BREWER DB: Ultrastructural localization of anionic and cationic ferritin in the rat glomerular basement membrane in protein-overload proteinuria. *J Pathol* 143:57–68, 1984